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בקשה לפטנט
Application For Patent

אני, (שם המבקש, מענו ולגבי גוף מאוגד – מקום ההתאגדות)
I (Name and address of applicant, and in case of body corporate-place of incorporation

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בעל אמצאה מכח הדין
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(בעברית) (Hebrew) תערובת להשמדת פטריות

(באנגלית) (English) **Fungicide Composition**

מבקש בזאת כי ינתן לי עליה פטנט
hereby apply for a patent to be granted to me in respect thereof.

*בקשה הלוקה- Division of Application	*בקשה פטנט מוסף- Addition Application for Patent	* דרישה דין קדימה Priority Claim		
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תערובת להשמדת פטריות

Fungicide Composition

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Fungicide Composition

The present invention relates to etheric oil emulsion for the control and treatment of a wide range of fungal plant pathogens (hereinafter "fungicide composition").

Increasing intensive agriculture favors the epidemic development of many new and previously unknown plant pests. This development has, in turn, necessarily led to the use of increasing dosages of chemicals, which negatively affects environmental health. On the other hand, registered pesticides are not always available to control pests effectively and reliably. Therefore, health and environmental considerations dictate the need for alternative methods of pest control, which can be promoted as possible strategies for inclusion in an Integrated Pest Management (IPM) program. IPM is a combination of crop protection practices, designed to maintain pests below a designated economic threshold; these practices fall into the categories of chemical, cultural, biological and host-plant resistance.

The control and treatment of plant diseases in greenhouses and field-grown horticultural crops is a serious problem in agriculture. So far powdering or spraying compositions of mineral source, e.g. sulfur, cuprous hydroxide, calcium polysulfate etc. or compositions based on detergents or oils have been mainly used. However, the use of said compositions is very unsatisfactory as they have many drawbacks.

From the Australian Journal of Experimental Agriculture, 39, 1999 Csiro 1999 it was shown that tea tree oil inhibits certain fungi. The treatment was quite satisfactory as it killed the fungi to a large extent - mainly fungi that attack human- While in plants it caused phytotoxicity to attacked plants.

It was thus desirable to develop a composition for the control and treatment of a wide range of fungal plant pathogens, e.g. which would not use any of the above known compositions, e.g. mineral oils, detergents and/or fats. However, it may comprise tea tree oil as one of its components. It should be easy, safe and relatively clear to prepare said compositions, and they should be convenient to use and be stable.

The present invention thus consists in a fungicidal emulsion comprising tea tree oil and a water emulsion in which the emulsifier is a water solution of a reaction product of a high molecular weight organic fatty acid and an alkali or ammonium compound.

The tea tree oil may have various compositions for example as shown in

Table 1

	%		%		%
Terpinen 4 ol	41.01	Alpha Terpinene	10.42	Aromadendrene	1.30
1,8 – Cineole	2.23	Terpinolene	3.63	Viridiflourene	1.05
Limonene	0.95	Gamma Terpinene	22.92	Delta Cadinene	1.18
Alpha Pinene	2.85	Alpha Terpineol	2.78	Globulol	0.28
Para Cymene	2.47	Sabinene	0.13	Viridiflourol	<0.1

There may be part of the composition according to the present invention various additional etheric oils, e.g. lavender oil, pine oil, manuka oil, kanuca oil, eucaliptuse oil, bergamot oil, clove oil, lemon oil etc.

The high molecular weight fatty acid is advantageously saturated or unsaturated with C > 12 atoms in the molecule, e.g.

a. tall oil acids, naftenic acids, rosin acids, and mixture of these acids etc.;

b. saturated fatty acid, e.g. lauric acid, myristic acid, palmitic acid, stearic acid, arahinoic acid, behenic acid, lignocerinicacid, etc.;

c. unsaturated fatty acid, e.g. decenoic, dodecenoic, palamitinoleic, oleic, linolenic, arahidonic, undecelenic, sorbic, recionoleic, etc. and alkali hydroxides, carbonates or bicarbonates.

For the preparation of the salts are advantageously used suitable sodium, potassium or ammonium compounds, e.g. hydroxides, carbonates or .bicarbonates.

The aqueous emulsion according to the present invention comprises advantageously 0.01% - 10 %, preferably 0.1%-1.5% tea tree oil, 0.02% - 10 %, e.g. of the salt, advantageously 0.1%-1%, the remainder being water.

When an additional etheric oil are present these are preferably added 0.01%-5%, advantageously 1%-5%.

The emulsion according to the present invention is advantageously prepared as water solution of alkaly hydroxide or carbonate (bicarbonate), mixing the solution obtained with an organic acid in liquid form and then, tea tree oil optionally combined with another etheric oil, is added. The mixing is suitably performed under continuous admixing in order to receive a homogenous composition.

Freshly prepared salts solution in water give good emulsification of tea tree oil in various concentrations. However, it is possible to use industrially prepared alkali salts of organic acid in powder or granulated form, to dissolve the salt obtained in hot water and to use the received solutions for the emulsification of the tea tree oil.

The suggested formulation is active against the following fungal pathogens, diseases and agricultural crops, as shown in Table 2.

Table 2

Pathogen	Disease	Crop
Oomycetes	**Downy mildews and Late blight**	**Grape, cucurbits, tomato, potato**
Phytopthora infestans	Late blight	Tomato, Potato
Plasmopara viticula	Downy mildew	Grape
Pseudoperonospora cubensis	Downy mildew	Cucurbits
Ascomycetes	**Powdery mildews**	**Grape, cucurbits, pepper, tomato**
Uncinula necator		
Spaerotheca fuliginea		
Levillula taurica		
Basidiomycetes	Rust Diseases	Roses
Tranzschelia discolor	Rust	Prunes, plums, peaches
Fungi imperfecti	**Alternaria**	**Various crops**
Alternaria solani	Early blight	Tomato, Potato
Alternaria alternata	Leaf and fruit decays and spots	Various crops
Aspargilus niger	Decays and spots	Various crops
Cladosporium spp.	Leaf spots, decays	Various crops (tomato, apple)
Penecillium spp.	Decays	Various crops - Citrus
Penicillium italicum	Decays	
Penicillium digitatum	Decays	
Botrytis cinerea	Fruit rots and decays	Various crops: vegetables flowers, grapes, etc.
Stemphillium spp.	Leaf spots	Various crops
Trichoderma		Various crops
Fusarium spp.	Decays, rots	Various crops

In general, fungal pathogens attack all parts of the plant such as:

flowers, fruits, stems, leaves, roots, tubers, bulbs, depends on each pathogen and host.

The present invention will now be illustrated with reference to the following Examples without being limited by them.

Example 1:

300 g of naftenic acid are mixed with 160 g of a 25% solution of NaOH in water during 60 minutes at 70°C. To the reaction product obtained is add 316 g tea tree oil with continuous stirring until full homogenization is obtained.

From the composition obtained which contains 50% tea tree oil, it is possible to prepare by adding water under continuous mixing a stable tea tree oil emulsion, comprising from 0.001% to 49.9% of oil.

Example 2:

Into a 25% water solution of 300 g KHCO3 is added under continues mixing 400 g of melted stearic acid at 75°C. After mixing during 30 minutes was added slowly a mixture 500 g of tea tree oil and of 200 g of lavender oil. All compounds were added until full homogenization was obtained.

From the received composition it is possible to prepare the required emulsions having from 0.001% to 49.9% w etheric oils.

Example 3:

30 g Na_2CO_3 were dissolved in 100 g water at 50°C. This solution was mixed with 120 g of tall oil acid (comprising 25% of rosin acid) during 30 minutes.

The received mixture was dissolved in 500 g of tea tree oil. The mixture was mixed to full homogenization.

This mixture obtained may be use for preparations stable emulsion of tea tree oil in various concentrations.

Example 4:

280 g of oleic acid was mixed with 85 g of a 20% ammonia solution at 60°C. To this composition was added 400 g of tea tree oil until full homogenization was

obtained. The composition obtained may be used for the preparation of stable tea tree oil emulsion at various concentrations.

Some emulsions were tested against certain fungal pathogens (TT in connection with the present invention means a tea tree oil composition according to the present invention).

The present invention shows that TT compositions are effective against a wide range of fungal pathogens belonging to *Oomycetes, Asomycetes, Basidiomycetes* and fungi Imperfecti. The Oomycetes group includes pathogens such as *Plasmopara viticola* in grapevine *Pseudoperonospora cubensis* in cucurbits, and *Phytophthora infestans* in tomato, which cause downy mildew and late blight. The Ascomycetes group includes the fungal pathogens such as *Uncinula necator, Sphaerotheca fuliginea,* and *Levillula taurica,* which causes the powdery mildews of grapevines, cucurbits and pepper and tomato, respectively. It also inhibited *Tranzschelia discolor* of the Basidiomycetes and several other pathogens including those belonging to Fungi imperfecti. These includes: *Alternaria alternata, Alternaria solani, Botrytis cinerea, Aspargilus niger, cladosporium spp, Penecillium spp., Penicillium italicum, Penicillium digitatum, Stemphillium spp, and Fusarium spp.*

Laboratory experiments revealed that TT compositions inhibited spore germination of and/or mycelial growth of these fungi in vitro. TT compositions showed prophylactic and local activity in intact plants and detached leaves. Foliar applications of TT compositions to field-grown grapevines and melons inhibited downy and powdery mildews development, respectively. The inhibitory effectiveness of TT compositions makes it well suited for integration into control programs against various diseases in agricultural organic-grown crops and as a replacement of sulfur and/or cupper.

The Experiments were performed by one of the following methods:

Spore germination test of downy mildews late blight, Alternaria and rust pathogens

Sporangial suspensions were mixed with various concentrations of TT (0-1%), and 0.1 mL droplets were transferred to depression glass slides (four slides for each concentration). Slides were incubated in moist Petri dishes at 20°C in darkness for 8 h. The percentages of sporangia releasing zoospores, and of zoospores producing germ tubes were counted under the microscope.

Effect on germination of conidia of powdery mildews pathogens

TT was dissolved and mixed with sterile distilled water to give a stock solution of a known concentration. TT was mixed with pre-autoclaved 1% water agar to give final concentrations of 0, 0.001, 0.01, 0.1 and 1.0%. Conidia were shaken onto glass slides previously coated with water agar containing TT. Slides were placed in Petri dishes containing wet filter paper and kept in the dark at 20°C for 16 h. The number of germinated conidia was counted under a microscope.

In vitro activity on mycelial growth of fungi.

Three-millimetre diameter agar disks bearing the tested fungus were taken from freshly growing colony on potato-dextrose-agar (PDA, 39 g of Difco potato dextrose agar in 1 l of distilled water) and placed on freshly amended with various concentrations of TT in 9-cm Petri dishes. Plates were incubated at 25°C in the dark for 6 days and colony diameter was recorded every two days. Three Petri dishes, each containing three inoculum disks, were used for each treatment concentration, and experiments were conducted twice.

In vivo (in planta) experiments

Plants were sprayed with TT at various concentrations (0-2%) on both surfaces, and 24 hours later were inoculated on the lower surface with sporangial suspension of *P. viticola* (grape downy mildew). The lower surface of each of six to eight attached leaves on each of six plants of each treatment was uniformLy sprayed with 2 mL of a sporangial suspension of 4x104 sporangia per mL, delivered from a glass chromatography sprayer. After inoculation, plants were covered with plastic bags, lightly sprayed on the inside with water, and were incubated at 19°C for 20 h in darkness. The plants were then uncovered and kept in a growth chamber for disease development. Nine days after inoculation, disease developed on each leaf of treated plant was evaluated. In some cases, plants were lightly sprayed with water, covered with plastic bags and incubated at 19°C for 24 h in darkness, to induce sporulation to determine sporangial production. Leaves were detached and the percent leaf area covered with sporangiophores and sporangia of *P. viticola* were visually estimated and recorded. The number of sporangia produced per square centimeter of leaf tissue was calculated as described above for leaf disks.

For powdery mildew inoculations, Conidia were shaken onto leaves previously treated with TT or with water. Plants were incubated in growth-room and percentage of leaf area covered with powdery mildew was assessed.

There were performed Experiments to evaluate the biological activity of the compositions according to the present invention against fungal pathogens.

The Experiments were performed with the following emulsion:
Oleic acid 150 g, Sodium hydroxide 20 g, Tea Tree oil 270 g, water 100 g in the concentrations as shown in the Examples.

Example 5: Control of powdery mildew (*Spaerotheca fuliginea)* in field-grown melon plants:

Treatment and Conc. (%)	% inhibition of infected leaf area as to control, Upper side of the leaf
Control	-------
0.25	50.0
0.5	72.5
1.0	84.5

Example 6: Effect on germination In Vivo - Grape powdery mildew pathogen:

Treatment (TT concentration, %)	% inhibition of conidial germination
Control – 0	------
0.1	100
0.01	96
0.001	63%

Example 7: Control of Grape downy mildew on plants:

Treatment (TT concentration, %)	% inhibition of infected leaf area
Control – 0	------
1.0	100
0.5	100
0.25	99

Example 8: Inhibition of mycelial growth of *Alternaria alternata* :

Treatment (TT Conc. %)	% inhibition of *A. alternata*
Control – 0	----
0.5	100
0.25	68
0.1	30

The results can be summarized as follows:

TT was found to be active against various stages of fungal pathogenesis of various fungal pathogens. For example, it controlled *Spaerotheca fuliginea* in field-grown melon plants (Example 5). It strongly inhibited germination of the grape powdery mildew fungus (*Uncinulla necator)* in which a concentration of 0.1% completely inhibited germination and at concentrations of 0.01 and 0.001 it provided 96 and 63% inhibition (Example 6).

TT was also effective in controlling foliar disease on leaves of potted plants. For example spraying of 0.25% of TT completely inhibited grape downy mildew (Example 7).

TT was also effective in inhibiting mycelial growth of some fungi (see Example 8).

CLAIMS

1. A fungicidal emulsion comprising tea tree oil and a water emulsion in which the emulsifier is a water solution of a reaction product of a high molecular weight organic fatty acid and an alkali or ammonium compound.
2. An emulsion according to Claim 1, comprising an additional etheric oil.
3. An emulsion according to Claim 2, in which the additional etheric oil is selected among lavender oil, pine oil, manuka oil, kanuca oil, eucalyptuse oil, bergamot oil, clove oil and lemon oil.
4. An emulsion according to any of Claims 1 to 3, in which the concentration of the tea tree oil is between 0.01% up to 10%.
5. An emulsion according to Claim 4, in which the concentration of the tea tree oil is between 0.1% to 1.5%.
6. An emulsion according to Claim 4 or 5, in which the concentration of the tea tree oil is 0.1% to 1.5%, the concentration of the salt is 0.1% to 1%, the remainder being water.
7. An emulsion according to any of Claims 1 to 6, wherein the alkali and ammonium compounds are selected among, sodium, potassium and/or ammonium hydroxides, carbonates and bicarbonates.
8. An emulsion according to any of Claims 2 to 7 wherein the concentration of the additional etheric oils is 0.01% to 5%.
9. A composition according to any of Claims 1 to 8, wherein the acid is selected among:
   a. tall oil acids, naftenic acids, rosin acids, and mixture of these acids;
   b. saturated fatty acid, e.g. lauric acid, myristic acid, palmitic acid, stearic acid, arahinoic acid, behenic acid, lignocerinicacid; and
   c. unsaturated fatty acid, e.g. decenoic, dodecenoic, palmitinoleic, oleic, linolenic, arahidonic, undecelenic, sorbic, recinoleic.
10. A composition as defined in Claim 1 substantially as herein described with reference to the Examples.

For the Applicant
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by: